

## WHAT IS CLAIMED:

1. A method for producing a plant artificial chromosome,  
comprising:
  - introducing a DNA fragment into a plant cell, wherein the
  - 5 DNA fragment comprises a selectable marker;
  - growing the cell under selective conditions to produce cells  
that have incorporated the DNA fragment into their genomic DNA; and
  - selecting a plant cell that comprises a satellite artificial  
chromosome (SATAC).
- 10 2. The method of claim 1, wherein the DNA fragment is  
introduced into or adjacent to an amplifiable region of a chromosome in  
the cell.
3. The method of claim 2, wherein the amplifiable region  
comprises rDNA.
- 15 4. The method of claim 2, wherein the amplifiable region  
comprises heterochromatin.
5. The method of claim 1, wherein the DNA is introduced into  
pericentric heterochromatin in a chromosome of the cell.
6. The method of claim 1, wherein the plant cell is a tobacco,  
20 rice, maize, rye, soybean, Brassica napus, cotton, lettuce, potato, tomato  
or arabidopsis cell.
7. The method of claim 1, wherein the plant cell is a monocot  
or dicot cell.
8. The method of claim 1, wherein the plant cell is a plant  
25 protoplast.
9. The method of claim 1, further comprising, isolating the  
SATAC.
10. The method of claim 1, wherein the DNA fragment  
comprises a sequence of nucleotides that targets the fragment to the  
30 heterochromatic region of a chromosome.
11. The method of claim 10, wherein the targeting sequence of

nucleotides comprises satellite DNA.

12. A SATAC produced by the method of claim 1.
13. An isolated substantially pure plant satellite artificial chromosome (SATAC).
- 5 14. The SATAC of claim 13 that is a megachromosome, comprising about 50 to about 450 megabases (Mb).
15. The SATAC of claim 13, comprising about 250 to about 400 Mb.
16. The SATAC of claim 13, comprising about 150 to about  
10 200 Mb.
17. The SATAC of claim 13, comprising about 90 to about 120 Mb.
18. The SATAC of claim 13, comprising about 15 to about 60 Mb.
- 15 19. A plant cell containing an artificial chromosome, wherein the artificial chromosome is produced by the method of claim 1.
20. A plant cell containing the SATAC of claim 12.
21. The method of claim 1, wherein the SATAC is a megachromosome, and the method further comprises:  
20 introducing a fragmentation vector, whereby the megachromosomes in the cells are reduced in size,  
and identifying cells that contain SATACs that are about 15 to about 60 Mb.
22. The method of claim 1, wherein the SATAC is a  
25 megachromosome, and the method further comprises, exposing the cells to conditions, whereby cells that contain truncated megachromosomes are produced.
23. The method of claim 23, wherein the conditions are selected  
30 from among exposure to X-rays, growth in the presence of an agent that destabilizes base pairing in the chromosome.

24. The method of claim 23, wherein the agent is bromodeoxyuridine.

25. The method of claim 2, further comprising selecting a cell that comprises a satellite artificial chromosome (SATAC) that comprises  
5 about 15 to about 60 Mb.

26. A plant cell containing an artificial chromosome, wherein the artificial chromosome is produced by the method of claim 22.

27. The cell of claim 26, wherein the artificial chromosome is a SATAC comprising about 10 to about 60 Mb.

10 28. An isolated substantially pure satellite artificial chromosome (SATAC) of claim 13 that comprises about 10 to about 60 Mb.

29. The method of claim 23, further comprising isolating the SATAC from the cell.

15 30. The method of claim 29, wherein isolation is effected by:  
isolating metaphase chromosomes;  
distinguishing SATACs from endogenous chromosomes; and  
separating the SATACs from endogenous chromosomes.

20 31. The method of claim 30, wherein:  
the SATACs are distinguished from endogenous chromosomes by  
staining the chromosomes with DNA sequence-specific dyes; and  
separation is effected by flow cell sorter.

32. A method for producing an artificial chromosome,  
comprising:

25 introducing a DNA fragment into a plant cell, wherein the  
DNA fragment comprises a selectable marker,  
growing the cell under selective conditions to produce cells  
that have incorporated the DNA fragment into their genomic DNA,  
selecting from among those cells, a cell that comprises a *de*  
*nov*o centromere.

30 33. The method of claim 32, further comprising isolating that cell  
with the chromosome that comprises the *de novo* centromere, and

growing the cell under conditions whereby a cell with a sausage chromosome is produced.

34. The method of claim 33, further comprising isolating the cell with the sausage chromosome; and growing the cell under conditions  
5 whereby a first SATAC is produced.

35. The method of claim 34, wherein the DNA fragment is introduced into or adjacent to an amplifiable region of a chromosome in the cell.

36. The method of claim 35, wherein the amplifiable region  
10 comprises rDNA.

37. The method of claim 35, wherein the amplifiable region comprises heterochromatin.

38. The method of claim 34, wherein the DNA is introduced into pericentric heterochromatin in a chromosome of the cell.

39. The method of claim 32, further comprising:  
15 introducing a fragmentation vector that is targeted to the first SATAC; growing the cells; and selecting a cell that comprises a second SATAC, wherein the second SATAC is smaller than the first SATAC.

40. The method of claim 39, wherein the selected cell has a  
20 dicentric chromosome comprising the *de novo* centromere.

41. The method of claim 39, wherein the selected cell has a formerly dicentric chromosome and a minichromosome comprising the *de novo* centromere.

42. The method of claim 39, wherein the selected cell has a  
25 formerly dicentric chromosome.

43. A method for producing a plant artificial chromosome, comprising

introducing a DNA fragment into a plant cell, wherein the DNA fragment comprises a selectable marker;

30 growing the cell under selective conditions to produce cells that have incorporated the DNA fragment into their genomic DNA;

selecting from among those cells a cell that has produced a dicentric chromosome; and

growing that cell under selective conditions, whereby a cell that contains a chromosome comprising a heterochromatic arm is produced.

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44. The method of claim 43, further comprising selecting the cell with the chromosome comprising the heterochromatic arm and growing it in the presence of an agent that destabilizes the chromosome.

45. The method of claim 44, further comprising identifying cells that contain a heterochromatic chromosome that is about 50 to about 400 Mb.

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46. The method of claim 43, wherein the DNA fragment is introduced into or adjacent to an amplifiable region of a chromosome in the cell.

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47. The method of claim 46, wherein the amplifiable region comprises rDNA.

48. The method of claim 46, wherein the amplifiable region comprises heterochromatin.

49. The method of claim 46, wherein the DNA is introduced into pericentric heterochromatin in a chromosome of the cell.

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51. A method for producing a transgenic plant, comprising introducing a satellite artificial chromosome (SATAC) into a protoplast.

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52. The method of claim 51, wherein the SATAC comprises heterologous DNA that encodes a gene product.

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53. The method of claim 51, wherein the SATAC is introduced by cell fusion, lipid-mediated transfection by a carrier system, microinjection, microcell fusion, electroporation, microprojectile bombardment, nuclear transfer or direct DNA transfer.

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54. A transgenic plant produced by the method of claim 51.

<sup>54</sup>  
~~55.~~ The transgenic plant of claim <sup>53</sup>~~54~~ that is tobacco, rice, maize, rye, soybean, Brassica napus, cotton, lettuce, potato, tomato or arabidopsis.

- <sup>55</sup>  
~~56.~~ A method of producing a transgenic plant, comprising:
- 5        introducing a DNA fragment into a first cell, wherein the DNA fragment comprises a selectable marker;
- growing the first cell under selective conditions to produce cells that have incorporated the DNA fragment into their genomic DNA; and
- 10        selecting a cell that comprises a minichromosome that is about 10 Mb to about 50 Mb that comprises the selectable marker and euchromatin; and
- isolating the minichromosome and introducing it into a plant cell.
- 15        <sup>56</sup>~~57.~~ The method of claim <sup>55</sup>~~56~~, wherein the first cell is a plant or animal cell.
- <sup>57</sup>~~58.~~ The method of claim <sup>55</sup>~~56~~, further comprising:
- after selecting the cell, introducing DNA encoding a gene product or products into the cell; and
- 20        growing the cell under selective conditions, whereby cells comprising minichromosomes comprising the DNA encoding the gene product(s) are produced.
59. A method of producing a transgenic plant, comprising:
- introducing a DNA fragment into a first cell, wherein the
- 25        DNA fragment comprises a selectable marker;
- growing the cell under selective conditions to produce cells that have incorporated the DNA fragment into their genomic DNA; and
- selecting a cell that comprises a satellite artificial chromosome (SATAC); and
- 30        isolating the SATAC and introducing it into a plant or animal cell.

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50. The method of claim 59, wherein the first cell is a plant or animal cell.
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61. The method of claim 58, wherein the first cell is a mammalian cell.
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62. The method of claim 59, further comprising:  
after selecting the cell, introducing DNA encoding a gene product or products into the cell; and  
growing the cell is under selective conditions, whereby cells comprising SATACS that comprise the DNA encoding the gene product(s) are produced.
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63. A method for cloning a centromere from a plant, comprising:  
preparing a library of DNA fragments that comprise the genome of the plant;  
introducing each of the fragments into mammalian satellite artificial chromosomes (SATACs), wherein:  
15 each SATAC comprises a centromere from a different species from the selected plant, and a selectable marker;  
introducing each of the SATACs into the cells and growing the cells under selective conditions;  
20 identifying cells that have a SATAC; and  
selecting from among those cells any that have a SATAC comprising a centromere that differs from the centromeres in the original SATAC.
- 63  
64. A SATAC of claim 14, comprising a sequence of nucleotides set forth in any of SEQ ID Nos. 18-24.
- 25  
64  
65. A SATAC of claim 13, comprising a sequence of nucleotides set forth in any of SEQ ID Nos. 18-24.
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66. A method for producing a transgenic plant, comprising introducing a satellite artificial chromosome (SATAC) into a plant cell; and  
30 culturing the cell under conditions whereby a plant is generated.

<sup>66</sup>  
~~67~~. The method of claim <sup>65</sup>~~66~~, wherein the SATAC is a mammalian artificial chromosome or a plant artificial chromosome.

<sup>67</sup>  
~~68~~. The method of claim <sup>61</sup>~~62~~, wherein the SATAC is introduced by protoplast fusion, microinjection, microcell fusion, lipid-mediated gene transfer, electroporation, microprojectile bombardment, nuclear transfer or  
5 direct DNA transfer.

<sup>68</sup>  
~~69~~. A method for producing a gene product(s), comprising introducing a satellite artificial chromosome (SATAC) of claim 1 into a cell; and culturing the cell under conditions whereby the gene product(s)  
10 is (are) expressed.

<sup>69</sup>  
~~70~~. The method of claim <sup>68</sup>~~69~~, wherein the gene product is produced by expression of a series of genes that encode proteins that comprise a metabolic pathway; and the SATAC comprises each of these genes.

<sup>70</sup>  
~~71~~. The method of claim 7, wherein isolation is effected by:  
15 isolating metaphase chromosomes;

distinguishing SATACs from endogenous chromosomes; and separating the SATACs from endogenous chromosome.

<sup>71</sup>  
~~72~~. The method of claim <sup>70</sup>~~71~~, wherein:  
20 the SATACs are distinguished from endogenous chromosomes by staining the chromosomes with DNA sequence-specific dyes; and separation is effected by flow cell sorter.

<sup>72</sup>  
~~73~~. A method for producing a transgenic plant, comprising introducing a satellite artificial chromosome (SATAC) of claim 13 into a  
25 plant cell; and culturing the cell under conditions whereby a plant is generated.

<sup>73</sup>  
~~74~~. A method for producing a gene product(s), comprising introducing a satellite artificial chromosome (SATAC) of claim 13 into a cell; and culturing the cell under conditions whereby the gene product(s)  
30 is (are) expressed.